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# Role of secreted glyceraldehyde-3-phosphate dehydrogenase in the infection mechanism of enterohemorrhagic and enteropathogenic *Escherichia coli*: Interaction of the extracellular enzyme with human plasminogen and fibrinogen

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**Abstract**

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (EC 1.2.1.12) is an anchorless, multifunctional protein displayed on the surface of several fungi and Gram-positive pathogens, which contributes to their adhesion and virulence. To date a role for extracellular GAPDH in the pathogenesis of Gram-negative bacteria has not been described. The aim of this study was to analyze the extracellular localization of GAPDH in enterohemorrhagic (EHEC) and enteropathogenic (EPEC) *Escherichia coli* strains and to examine its interaction with host components that could be related to the infection mechanism. Recombinant *E. coli* GAPDH was purified and polyclonal antibodies were obtained. Western blotting and immunoelectron microscopy showed that GAPDH is located on the bacterial surface and released to the culture medium of EHEC and EPEC strains. GAPDH export in these Gram-negative pathogens depends on the external medium, is not mediated by vesicles and leads to an extracellular active enzyme. Non-pathogenic *E. coli* strains do not secrete GAPDH. Two-dimensional electrophoresis analysis showed that in *E. coli* GAPDH is present at least in two major forms with different isoelectric points. Of these forms, the more basic is secreted. Purified GAPDH was found to bind human plasminogen and fibrinogen in Far-Western blot and ELISA-based assays. In addition, GAPDH remained associated with colonic Caco-2 epithelial cells after adhesion of EHEC or EPEC. These observations indicate that exported GAPDH may act as a virulence factor which could contribute to EHEC and EPEC pathogenesis. This is the first description of an extracellular localization for this enzyme, with a function other than its glycolytic role in Gram-negative pathogens.


*Keywords:* Extracellular glyceraldehyde-3-phosphate dehydrogenase; Enterohemorrhagic *Escherichia coli*; Enteropathogenic *Escherichia coli*; Infection mechanisms; Plasminogen; Intestinal epithelial cells

*Abbreviations:* BSA, bovine serum albumin; EHEC, enterohemorrhagic *Escherichia coli*; EPEC, enteropathogenic *Escherichia coli*; DMEM, Dulbecco's modified Eagle's medium; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GST, glutathione-*S*-transferase; LB, Luria–Bertani broth; MALDI-TOF, matrix-assisted laser desorption/ionization-time-of-flight; MS, mass spectrometry; PGPase, phosphoglycolate phosphatase; PVDF, polyvinylidene difluoride; SDS-PAGE, sodium dodecyl sulphate- polyacrylamide gel electrophoresis; TCA, trichloroacetic acid

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## 1. Introduction

A large number of recent reports have shown that classical cytoplasmic housekeeping enzymes with no detectable secretion or retention signal are present on the surface of microbial pathogens, where they exert functions related to the adhesion and/or virulence of the pathogen (Pancholi & Chhatwal, 2003). However, how these enzymes are exported and exposed on the surface is still unknown. Most descriptions of the expression of cytoplasmic housekeeping enzymes on the microbial surface deal with fungi and Gram-positive pathogens, whereas few examples have been reported for Gram-negative bacteria.

Among the housekeeping proteins with extracellular localization in pathogens there are several glycolytic enzymes, such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH; EC 1.2.1.12). The localization of GAPDH on the surface of a pathogen was first described in Gram-positive bacteria. In fact, two research groups simultaneously described this finding in group A *streptococci* (Lottenberg et al., 1992; Pancholi & Fischetti, 1992). Since then, several reports in yeast and other Gram-positive pathogens showed that extracellular GAPDH interacts with various host components, including plasminogen, cytoskeleton proteins (actin, myosin), or extracellular matrix proteins such as fibronectin (Gozalbo et al., 1998; Pancholi & Fischetti, 1992; Seifert, McArthur, Bleiweis, & Brady, 2003; Schaumburg et al., 2004). Recently, the membrane protein uPAR/CD87 in pharyngeal cells has been identified as a receptor for surface GAPDH in *S. pyogenes* (Jin, Song, Boel, Kochar, & Pancholi, 2005). These interactions contribute to the adhesion of the pathogen and to its virulence, and thus play a role in the mechanism of pathogenesis.

The possible extracellular localization of GAPDH and its role in Gram-negative pathogens has been poorly studied. In *Enterobacteriaceae*, GAPDH has been identified as an outer membrane protein in *Edwarsiella tarda* and the recombinant enzyme has been used to develop a vaccine against this pathogen (Kawai, Liu, Ohnishi, Oshima, 2004; Liu, Oshima, Kurohara, Ohnishi, & Kawai, 2005). In enteropathogenic *Escherichia coli* (EPEC), a 39 kDa protein with an N-terminal sequence displaying significant similarity to GAPDH was observed among some secreted proteins in the culture medium (Kenny & Finlay, 1995). In *E. coli*, GAPDH is encoded by two genes, *gapA* and *gapC*. Many *E. coli* K-12 strains routinely used as standards in experimental research have accumulated mutations in *gapC*, generating stop codons which result in a truncated, non-functional protein (Espinosa-Urgel, & Kolter, 1998; Hidalgo, Limón, & Aguilar, 1996). However, in natural isolates or pathogenic *E. coli* strains the *gapC* open reading frame is complete, although no information is available on its expression.

EPEC and enterohemorrhagic *E. coli* (EHEC) are closely related human pathogens which secrete many extracellular proteins and are distinguished from other pathogenic *E. coli* strains by their ability to produce a characteristic histopathological feature, known as attaching and effacing lesions, on the mucosa (Donnenberg & Whittam, 2001).

Here we examined GAPDH as a secreted protein in these Gram-negative pathogens. We show that in EHEC and EPEC, GAPDH is an extracellular protein that binds human plasminogen and fribinogen, and interacts with intestinal epithelial cells. This finding indicates a role of extracellular GAPDH in the infection mechanism.

## 2. Materials and methods

### 2.1. Bacterial strains, plasmids and growth conditions

The *E. coli* strains and plasmids are listed in Table 1. Bacterial cells were routinely grown at 37 °C in Luria–Bertani broth (LB). The *gapA* mutant strain W3CG was grown in minimal medium supplemented with malate and glycerol as carbon sources (Ganter & Plückthun, 1990). Where indicated, the cells were grown without shaking at 37 °C in Ham's F-12, Dulbecco's modified Eagle's medium (DMEM) (GIBCO), or minimal medium (Boronat & Aguilar, 1979) supplemented with 20 mM glucose, in a $CO_2$ incubator. Growth was monitored by measuring the optical density at 600 nm ($OD_{600}$). When required, tetracycline (12.5 μg/ml) or ampicillin (100 μg/ml) was added to the medium.

### 2.2. Caco-2 cell culture, infection conditions, and fractionation of infected Caco-2 cells with Triton X-100

Caco-2 cells were grown at 37 °C in a 5% $CO_2$ atmosphere in DMEM supplemented with nonessential amino acids, 25 mM HEPES, 10% fetal calf serum, penicillin (100 U/ml) and streptomycin (100 μg/ml).

Before infection, confluent monolayers of Caco-2 cells were washed three times in PBS (140 mM NaCl, 2.7 mM KCl, 10 mM $Na_2HPO_4$, 1.8 mM $KH_2PO_4$, pH 7.3), and maintained in DMEM without serum or antibiotics. EHEC 86-24h11 or EPEC E2348/69 were grown in LB overnight at 37 °C, and then the culture was diluted